DEVELOPMENT OF DISCRIMINATING CONCENTRATIONS FOR INSECTICIDE RESISTANCE MONITORING IN THE SOYBEAN LOOPER

R.N. Mascarenhas, D.J. Boethel and B. R. Leonard
Department of Entomology
Louisiana State University
Agricultural Center
Baton Rouge, LA

Abstract

Discriminating concentrations of several standard and experimental insecticides were determined for an insecticide susceptible strain of soybean looper using an insecticide diet overlay bioassay. These concentrations were used to evaluate the relative susceptibility of field and F1 generations of three field-collected strains of soybean looper larvae. Field strains exhibited significantly higher percent survival compared to the susceptible reference strain (USDA) when exposed to Ambush®, Condor XL® and Larvin® as larvae directly from the field or as F1 laboratory-reared larvae. Soybean looper larvae collected from Bt-cotton had higher survival when exposed to Condor XL® than the USDA larvae. Larvae from field strains exposed to the discriminating concentration of Pirate® and Proclaim® did not exhibit significantly higher survival than that of the reference strain. In the Proclaim® bioassays, larval survival for two field strains of the field generation was significantly lower than that of the reference strain. In the Tracer bioassays, two strains in the field generation bioassays and one strain in the F1 generation bioassay had survival significantly higher than the USDA strain. These differences may have been due to natural variation in the soybean looper population, but need further investigation.

Introduction

The soybean looper, *Pseudoplusia includens* (Walker), is an extremely important defoliating pest of soybean grown in the southeastern United States. Soybean is the preferred host, but the larvae can be found feeding on a multitude plants including cotton (Canerday and Arant 1966). Although the soybean looper is considered to be only an occasional pest of cotton, the crop is considered to have a great impact on this pest's population dynamics. For example, it serves as a source of nectar for the adults (Burleigh 1972, Jensen et al. 1974) and as a probable site for development of insecticide resistance (Felland et al. 1990, Leonard et al. 1990, Thomas and Boethel 1994). Data collected by Canerday and Arant (1966) in Alabama and Hensley et al.(1964) in Louisiana indicate that soybean loopers comprise 19.1% and 13.7%, respectively, of the looper species found in cotton during the growing season. However, pyrethroids applied to cotton during the season can remove virtually all other looper species while having little effect on soybean looper populations. This allows soybean loopers to become the predominant Plusiinae species in cotton late in the season (Boethel et al. 1992).

In the early 1980's, permethrin was the standard insecticide used by soybean growers for soybean looper control. However, during the 1987 growing season, its efficacy began to decline in Georgia (Herzog 1988), Mississippi (Felland et al. 1990), and Louisiana (Leonard et al. 1990), and shortly after, resistance was documented in these areas. Because soybean in the southeastern United States is rarely treated more than once per growing season, it is highly unlikely that this selection pressure occurred due to insecticide applications made against soybean looper on soybean (Thomas and Boethel 1994). Rather, this resistance probably occurred due to control measures directed toward insect pests in cotton, or from insecticide applications made to soybean looper populations at their point of origin. Soybean looper is a migratory insect and is not known to overwinter in Louisiana. Populations are thought to migrate annually from portions of Central and South America and the southernmost areas of Texas and Florida. It is these source populations which are exposed to intensive insecticide applications and whose survivors eventually migrate to Louisiana (Boethel et al. 1992).

Soybean looper resistance to permethrin has been studied extensively. Baseline dosage-mortality data for this pest have been collected for several pyrethroid insecticides (Leonard et al. 1990, Mink and Boethel 1992). In 1992, Mink and Boethel developed a diagnostic technique for evaluating permethrin resistance in the soybean looper by exposing larvae to permethrin-coated vials. This technique provided a relatively quick and simple method for determining permethrin resistance levels of soybean looper populations from different geographic regions. In addition, the resistance mechanisms involved (Rose et al. 1990, Thomas and Boethel 1994) and the manner in which pyrethroid resistance is inherited have been determined (Thomas and Boethel 1995). Cumulatively, these data have provided researchers with valuable information to help avoid further losses of insecticide chemistries to resistance.

Several new chemistries with unique modes of action have been developed and may soon become available for soybean looper control in both cotton and soybean. These compounds include chlorfenapyr or Pirate® (American Cyanamid, Princeton, NJ), emamectin benzoate or Proclaim® (Merck & Companay, Inc., Three Bridges, NJ) and spinosad or Tracer® (DowElanco, Indianapolis, IN). Pirate® and Proclaim® are primarily stomach poisons and require ingestion of the toxin to be active. Although Tracer® provides some contact activity, it is most toxic when administered orally (Sparks 1995). Although these chemistries have modes of action which differ from pyrethroids, it is imperative that as much information

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 2:1021-1024 (1997)
National Cotton Council, Memphis TN

concerning their toxicity to pests such as the soybean looper be evaluated in order to avoid future resistance development.

The objective of this study was to evaluate these experimental insecticides, as well as standards that have been and are currently being recommended for soybean looper control in Louisiana, against laboratory and field strains of soybean looper. This was accomplished by establishing discriminating concentrations for each compound using an insecticide diet overlay bioassay. This type of information should prove useful for soybean looper resistance monitoring by providing a historical database on the activity of these insecticides and may aid in prolonging their use as effective control measures in both soybean and cotton.

Methods and Materials

Insects. Soybean loopers were collected from three locations in Louisiana from soybean fields during August of 1996. The sampling locations included Jeanerette (JEAN) in Iberia Parish; Morganza (MORG), in Pointe Coupee Parish; and Winnsboro (WINN), in Franklin Parish. In addition, transgenic Bt cotton was sampled for soybean loopers at two locations: Morganza (Bt-MOR) and Winnsboro (Bt-WIN). Larvae were transported to the laboratory and placed in 10 oz paper rearing cups (15 larvae per cup) containing pinto bean-wheat germ diet (Thomas et al. 1993). Larvae were allowed to feed for 24 hours on artificial diet, and 3rd to 5th instars of the field generation were selected for subsequent bioassays for all locations (except those collected from transgenic Bt cotton). In addition to testing individuals from the field generation, a number of larvae from each field strain remained in colony and were reared to the F1 generation. These individuals were tested when larvae reached 3rd, 4th and 5th instars. In addition, an insecticide-susceptible strain of soybean looper (USDA) was obtained from the USDA-ARS Southern Insect Management Laboratory at Stoneville, MS and served as the reference strain. Rearing procedures for the USDA strain were similar to those used for the field strains.

Bioassays. Artificial diet overlay bioassays were conducted to evaluate the effects of formulated Ambush® (permethrin-25.6% ai; Zeneca Agricultural Prod., Wilmington, DE); Condor OF® (*Bacillus thuringiensis* var. *kurstaki*-7.5% ai; Ecogen, Inc., Langhorne, PA); Larvin® (thiodicarb-32.5% ai; Rhone-Poulenc Ag. Co., Research Triangle Park, NC); Pirate® (chlorfenapyr-36% ai; American Cyanamid, Princeton, NJ); Proclaim® (emamectin benzoate-2.15% ai; Merck Research Labs, Rahway, NJ) and Tracer® (spinosad-44.2% ai; DowElanco, Indianapolis, IN) on 3rd-5th instar (20-125 mg (Shour and Sparks 1981)) field-collected and laboratory-reared soybean loopers. Stock solutions (10,000 ppm in distilled water) were prepared based upon percent active ingredient (ai). Dilutions were made using distilled water to obtain the discriminating concentration for each insecticide. Discriminating concentrations were dependent on the compound tested, but were those concentrations which killed approximately 90-95% of the individuals tested from the susceptible strain. The field generation of each strain of soybean looper collected from soybean (JEAN, MORG, WINN) and the susceptible reference strain (USDA) were exposed to diet treated with the discriminating concentration of each insecticide as well as a distilled water control. The same procedure was conducted on all field strains (strains listed above as well as Bt-MOR and Bt-WIN) using the F1 generations to ensure that mortality was not due to factors other than insecticide exposure.

Three mls of liquid pinto bean-wheat germ diet were pipetted into plastic 30 ml diet cups. The diet was allowed to cool and gel, and 100 µl of the discriminating concentration of insecticides were pipetted into each cup, and diet cups were rolled and shaken slightly to evenly distribute the insecticide across the diet surface and allowed to dry for 1 h. One larva was placed in each cup, and the cups were capped. Mortality was recorded 72 h after treatment for each strain and insecticide evaluated, and larvae were considered dead if they did not respond to prodding. All data were corrected for control mortality using Abbott's (1925) formula and analyzed using specific linear contrasts (SAS Institute 1988).

Results and Discussion

The responses of the field-collected and F1 generations of the soybean looper strains evaluated are listed as percent survival in Tables 1 and 2, respectively. The discriminating concentration for Ambush® was 5 ppm, and all strains from the field and F1 generations had survival levels significantly higher than that of the susceptible reference strain. However, the highest percent survival was observed in the field and F1 generations of the MORG and WINN strains, which were collected in areas of cotton production. These results confirm previous studies that documented increased soybean looper resistance to pyrethroids in areas where cotton and soybean are grown in close proximity (Leonard et al. 1990, Felland et al. 1990, Mink and Boethel 1992) as compared to areas where soybean is predominately produced. Vial data using the discriminating dose of permethrin, from the 1996 growing season indicated that soybean looper populations from Jeanerette, Morganza and Winnsboro exhibited 60, 56, 71% survival, respectively (D. J. Boethel, unpublished data).

In the Condor XL® bioassays, the discriminating concentration used for all strains was 130 ppm. As in the Ambush® bioassays, all of the field strains from both generations had significantly higher percent survival than the USDA strain (Tables 1 and 2). The F1 larvae of soybean looper collected from Bt-cotton in Morganza (Bt-MOR) and Bt-cotton in Winnsboro (Bt-WIN) exhibited the highest percent survival (73 and 65%, respectively) of all

the field strains tested. Baseline dosage-mortality studies were conducted using these two Bt-cotton strains comparing them to the USDA strains (R. N. Mascarenhas and D. J. Boethel, unpublished data). These studies revealed that the Bt-MOR and Bt-WIN strains had LC50s of 188.08 and 90.14 ppm, respectively, which was significantly higher than the LC50 of 27.12 ppm observed in the USDA strain (Table 3). These data indicate reduced susceptibility of soybean looper populations to *Bacillus thuringiensis*. Although there have been reports of inconsistent results concerning soybean looper control with *B. thuringiensis* insecticides in Louisiana, no documented field control failures have been reported at this time with these products. However, for several years, the Louisiana Cooperative Extension Service's insect control recommendations have indicated that more consistent control of soybean loopers resulted from the use of higher labeled rate of recommeded Bt products (Baldwin et al. 1996)

The discriminating concentration used in the Larvin® bioassays was 1300 ppm. Significant differences were observed in survival between the USDA (5%) strain and the MORG (28%) and WINN (20%) strains in the field generation assays (Table 1). The differences in percent survival observed between the USDA and field strains became less evident in the F1 generation assays; however, there remained significant differences between the USDA strain and the JEAN (14%) and MORG (17%) strains (Table 2). These differences in survial may indicate an area where soybean looper populations are more tolerant to Larvin®, but, this insecticide is still providing excellent control of soybean looper in Louisiana (Mascarenhas et al. 1996). In other states such as Alabama (Sullivan 1992) and South Carolina (Sullivan and Chapin 1990), growers have had to use higher rates of Larvin® that than recommended in Louisiana to adequately control soybean loopers. For this reason, researchers and growers should be aware of the changes observed in the response of soybean looper populations in Louisiana to this insecticide.

In the Pirate® bioassays, a discriminating concentration of 60 ppm was used. There were no significant differences in the percent survival between the USDA (6%) strain and any of the field strains in the field or F1 generation assays (Tables 1 and 2). These results were not surprising because Pirate® has not been used commercially for soybean looper control.

The discriminating concentration determined for Proclaim® was only 5 ppm, which was the lowest concentration developed for all the experimental insecticides evaluated. The percent survival observed in the USDA strain was 5%, and the only strains with survival significantly different from the USDA strain were the JEAN (0%) and WINN (0%) strains in the field generation. However, both of these strains exhibited survial lower than that of the USDA strain (Table 1). There were no significant differences among strains in the F1 generation bioassays (Table 2). As with Pirate®, Proclaim® has not been labeled for use in soybeans, so the results from these assays were expected.

Finally, in the Tracer® bioassays, a discriminating concentration of 60 ppm was obtained. In the field generation bioassays, the JEAN (8%) and WINN (10%) strains both exhibited percent survival significantly higher that that seen in the USDA (2%) strain (Table 1). When the F1 generation was tested, only the JEAN (10%) strain had survival significantly higher than the USDA strain (Table 2). As with the other experimental insecticides, this compound has not been applied commercially for soybean looper control, so these results are difficult to explain. In field trials in Louisiana, this compound has given very good control of soybean loopers (Mascarenhas et al. 1996).

In conclusion, data from this study indicate that the standard insecticides used in Louisiana for soybean looper control, Condor XL® and Larvin®, have variable activity against larvae exposed to the discriminating concentrations. However, field data have indicated that these compounds are still providing adequate control of soybean loopers. The experimental compounds provided good activity against of most strains of soybean loopers evaluated and show promise as alternative insecticide chemistries for managing this pest.

The development of discriminating insecticide concentrations is important in detecting and monitoring resistance in field populations of pest insects such as the soybean looper. This type of research represents a proactive approach to combating insecticide resistance before it develops in the field to standard insecticides, such as Condor® and Larvin®, and before experimental products, such as Pirate®, Proclaim® and Tracer® are actually registered. If these concentrations can be determined prior to field control failures, researchers may be able to stay one step ahead of insect resistance development and perhaps extend the use of these insecticide chemistries as viable control measures.

References

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265-276.

Baldwin, J. L., D. J. Boethel and B. R. Leonard. 1996. Control of soybean insects, 1996. Louisiana Cooperative Extension Service Pub. No. 2211: pp. 16.

Boethel, D. J., J. S. Mink, A. T. Wier, J. D. Thomas, B. R. Leonard and F. Gallardo. 1992. Management of insecticide resistant soybean loopers (*Pseudoplusia includens*) in the southern United States. p. 68. *In* L. G. Copping, M. B. Green and R. T. Rees [eds.], Pest Management in Soybean. 1992. Essex, England.

Burleigh, J. G. 1972. Population dynamics and biotic control of the soybean looper in Louisiana. Environ. Entomol. 1: 290-294.

Canerday, T. D. and F. S. Arant. 1966. The looper complex in Alabama (Lepidoptera, Plusiinae). J. Econ. Entomol.

Felland, C. M., H. N. Pitre, R. G. Luttrell and J. L. Hamer. 1990. Resistance to pyrethroid insecticides in soybean looper (Lepidoptera: Noctuidae) in Mississippi. Environ. Entomol. 20: 498-506.

Hensley, S. D., L. D. Newsom and J. Chapin. 1964. Observations on the soybean looper complex of the noctuid subfamily Plusiinae. J. Econ. Entomol. 57: 1006-1007.

Herzog, G. A. 1988. Performance of pyrethroids in the southeastern region of the cotton belt, pp. 231-232. *In* Proceedings 1988 Beltwide Cotton Conference, National Cotton Council, Memphis, TN.

Jensen, R. L., L. D. Newsom and J. Gibbens. 1974. The soybean looper: Effects of adult nutrition on oviposition, mating frequency, and longevity. J. Econ. Entomol. 67: 467-470.

Leonard, B. R., D. J. Boethel, A. N. Sparks, Jr., M. B. Layton, J. S. Mink, A. M. Pavloff, E. Burris and J. Graves. 1990. Variations in soybean looper (Lepidoptera: Noctuidae) response to selected insecticides in Louisiana. J. Econ. Entomol. 83: 27-34.

Mascarenhas, R. N., B. J. Fitzpatrick and D. J. Boethel. 1996. Evaluation of selected experimental and standard insecticides against soybean looper and beet armyworm in northeast LA, 1995. Arthropod Mgt. Tests: 1996, 21: 292.

Mink, J. S. and D. J. Boethel. 1992. Development of a diagnostic technique for monitoring permethrin resistance in soybean looper (Lepidoptera: Noctuidae) larvae. J. Econ. Entomol. 85: 1056-1062.

Rose, R. L., B. R. Leonard, T. C. Sparks and J. B. Graves. 1990. Enhanced metabolism and knockdown resistance in a field versus laboratory strain of the soybean looper (Lepidoptera: Noctuidae). J. Econ. Entomol. 83: 672-677.

SAS Institute Inc. 1988. SAS/STAT user's guide, version 6.03, [ed.] SAS Institute, Cary, NC. 1028 pp.

Shour, M. H. and T. C. Sparks. 1981. Biology of soybean looper, *Pseudoplusia includens*: Characterization of last stage larvae. Ann. Entomol. Soc. Am. 74: 521-535.

Sparks, T. C., H. A. Kirst, J. S. Mynderse, G. D. Thompson, J. R. Turner, O. K. Jantz, M. B. Hertlein, L. L. Larson, P. J. Baker, M. C. Broughton, J. D. Busacca, L. C. Creemer, M. L. Hubner, J. W. Martin, W. M. Nakatsukasa, J. W. Paschal and T. V. Worden. 1995. Chemistry and biology of the spinosyns: Components of spinosad (Tracer®), the first entry into DowElanco's naturalyte class of insect control products. pp. 692-696. *In* Proceedings 1996 Beltwide Cotton Conference, National Cotton Council, Nashville, TN.

Sullivan, M. J. 1992. Insecticides recommended for control of soybean loopers in the southern United States: Report of their effectiveness. pp. 35. *In* J. Hamer and H. N. Pitre [eds.], Proceedings of Soybean Looper Resistance Workshop. 1992. Nashville, TN.

Sullivan, M. J. and J. W. Chapin. 1990. Soybean looper resistance workshop. pp. 37. *In* J. Hamer and H. N. Pitre [eds.], Proceedings of Soybean Looper Resistance Workshops. 1990. Orange Beach, AL.

Thomas, J. D., J. S. Mink and D. J. Boethel. 1993. Diet influences in permethrin susceptibility and growth of soybean looper larvae (Lepidoptera: Noctuidae). J. Econ. Entomol. 86: 1236-1240.

Thomas, J. D. and D. J. Boethel. 1994. Synergism of insecticides in tests with resistant soybean looper larvae (Lepidoptera: Noctuidae) in the laboratory and field. J. Econ. Entomol. 87: 1416-1422.

Thomas, J. D. and D. J. Boethel. 1995. Inheritance of permethrin resistance in the soybean looper (Lepidoptera: Noctuidae). J. Econ. Entomol. 88: 1536-1541.

Table 1. Percent survival of different strains of soybean looper larvae (3rd-5th instars)from the field generation exposed to discriminating concentrations of selected standard and experimental insecticides.

	Soybean looper strain			
Insecticide	USDA	JEAN	MORG	WINN
Ambush®	4	61*	93*	94*
Condor XL®	5	47*	39*	46*
Larvin®	5	9	28*	20*
Pirate®	6	9	10	5
Proclaim®	5	0*	6	0*
Tracer®	2	8*	10*	3

* Significantly different from USDA ($P \leq 0.05$, Specific Linear Contrasts (SAS 1988).

Table 2. Percent survival of different strains of soybean looper larvae (3rd-5th instars)from the F1 generation exposed to discriminating concentrations of selected standard and experimental insecticides.

	Soybean looper strain					
Insecticide	USDA	JEAN	MORG	WINN	OR	BTWIN
Ambush®	4	53*	97*	100*	--	--
Condor XL®	5	20*	29*	37*	73*	65*
Larvin®	5	14*	17*	5	--	--
Pirate®	6	6	7	7	--	--
Proclaim®	5	3	1	5	--	--
Tracer®	2	10*	5	3	--	--

* Significantly different from USDA ($P \leq 0.05$, Specific Linear Contrasts (SAS 1988).

Table 3. Toxicity of Condor XL® (*Bacillus thuringiensis*) treated diet (in ppm) to third instar soybean looper (F1 generation) 72 h after treatment.

Strain	n	LC50 (95% CL)
USDA	250	27.12 (21.92-33.17)
Bt-MOR	250	188.08 (147.18-239.51)
Bt-WIN	250	90.14 (67.82-112.59)

Non-overlap of 95% confidence limits indicate a significant difference among strains.